DOCKET NO.: 287325US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF:	:
Hisashi TAKAHASHI, ET AL	: EXAMINER : SEAMAN, D.M.M.
SERIAL NO. : 10/572,742	:
FILED: March 21,2006	: ART UNIT: 1625

FOR: 8-CYANOQUINOLONECARBOXYLIC ACID DERIVATIVE

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

Sir:

Now comes Hisashi Takahashi who deposes and states that:

1. I am a graduate of Shizuoka College of Pharmacy, and received my Ph. D. degree in the field of Pharmaceutical Science, in the year 1990.

2. I have been employed by Daiichi Pharmaceutical Co. Ltd., for 18 years ~~in the field of~~ and then employed by Daiichi Sankyo Co. Ltd., for 2 years ~~,~~ in the field of Medicinal Chemistry.

3. I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same.

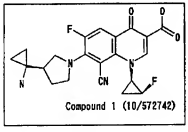
4. The following experiments were carried out by me or under my direct supervision and control.

5. To further illustrate that the present invention provides unexpected results as compared to structurally similar compounds disclosed in Saito (US 6,825,353), Ding (US 7,238,694), and the various cited Takemura references, the following experiments were performed to assess the antibacterial activities thereof and the correlation with hERG-potassium ion channel thereof were assayed according to the results of these experiments.

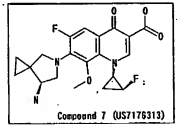
6. The following experiments were performed:

Experiment I

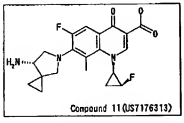
Takemura et al. (US 7,167,313) discloses an anti acid-fast agent, and provides evidence related to such anti acid-fast activities. But such evidence makes little sense in our investigation into antibacterial activities of the compounds. For that reason, an experiment was carried out in order to investigate antibacterial activities of the compounds described in Takemura et al., i.e., Compound 7 and Compound 11 disclosed therein. These compounds were compared with Compound 1 of the present invention whose antibacterial activities are already shown in Table 1 of the present application. The data obtained by this experiment are shown in Table 3 below, which includes the data of Compound 1 shown in Table 1 of the present application.



Compound 1 (10/572742)



Compound 7 (US7176313)



Compound 11(US7176313)

Table 3:

	US7176313		10/572742
	compound 7	compound 11	Compound 1
E. coli NIHJ	0.006	≤0.003	≤0.003
S. flexneri 2A 5503	0.006	0.006	≤0.003
P.vulquaris 08601	0.012	0.006	0.006
K. pneumoniae TYPE I	0.025	0.05	0.025
S.marcescens 10100	0.05	0.1	0.025
P. aeruginosa 32104	0.1	0.05	0.10
P. aeruginosa 32121	0.05	0.05	0.05
S.maltophilia IID 1275	0.1	0.2	0.20
S. aureus FDA 209P	0.006	0.006	≤0.003
S.epidermidis 56500	0.05	0.1	0.006
S. pyogenes G-36	0.05	0.05	0.006
E.gaevcalis ATCC 19433	0.1	0.2	0.025
S. aureus 870307	0.39	0.78	0.05
S. pneumoniae J24	0.025	0.025	0.006

As can be seen from Table 3, Compound 1 of the present invention exhibits surprisingly strong antibacterial activities against a wide spectrum of both Gram-positive and Gram-negative bacteria. In contrast, Compounds 7 and 11 of Takemura et al. show much weaker antibacterial activities, especially against Gram-positive bacteria, and these compounds are also extremely weak even against methicillin-resistant staphylococcus aureus (MRSA). Thus it appears that the presently claimed invention is not rendered obvious by Takemura et al.

Experiment II

The industrial applicability of a pharmaceutical product is commonly required to satisfy not only the requirement for pharmaceutical efficacy, but also the requirement for pharmaceutical safety. Therefore, a further experiment was carried out to investigate the possibility of adverse cardiac side-effects that might be caused by the dosage of a drug. Specifically, this experiment focuses on whether there is a correlation between the dosage of

a drug and the inhibition of hERG-potassium ion channel which is known as relevant to the prolongation of QT.

In this experiment, Compound 1 was used as the representative compound of the present invention, and thus was compared with the counterpart compounds described in the cited prior art.

Sito et al. (US 6,825,353) relates to a method for the efficient production of a 5-amino-8-methylquinolonecarboxylic acid derivative having an amino substituent as the 7-position substituent thereof, and to intermediate compounds to be used therein. This reference discloses a compound represented by formula (A) (DQ-113), which, it seems, is structurally similar to the presently claimed compounds. Therefore, this compound was selected as a counterpart compound to be compared with Compound 1 of the present invention.

Takemura et al. (US 6,448,266) discloses a compound having a cyclobutylamine at the position 8 of its quinolone ring. This compound is exemplified in Examples 5, 6 and 7, which, it seems, are structurally similar to the presently claimed compounds. Therefore, these compounds were also selected as counterpart compounds to be compared with Compound 1 of the present invention.

Takemura et al. (US 6,184,388), which is a continuation of US 6,121,285, discloses a compound having an aminocycloalkylpyrrolidine substituent at the position 7 of its quinolone ring. This compound is exemplified in Examples 15 and 16, which, it seems, are structurally similar to the presently claimed compounds. Therefore, these compounds were also selected as counterpart compounds to be compared with Compound 1 of the present invention. It is noted especially that Compound 15 and Compound A share the same structure.

7. Particulars of this experiment as an assay of inhibitory effects on hERG current by use of the automated patch clamp system Ionworks Quattro™ are as follows:

Method

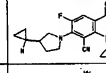
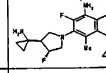
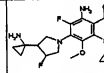
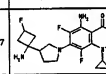
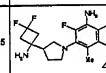
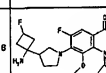
An assay was carried out according to the patch clamp technique, in which a recombinant CHO-K1 cell line expressing the human ERG (ether-a-go-go related gene) potassium channel was used as a cell line, and PPC (Population Patch Clamp™) was employed as a plate. The electric current that runs through the hERG channel of the cell surface was measured by the perforated patch using amphotericin. The holding voltage was adjusted at -80 mV, and the implementation of depolarization pulse was continued at +40 mV (or +45 mV) for 2 second, followed by the implementation of repeated polarization pulse at -50 mV (or -45 mV) for 2 second. The maximum current that was observed at 20 to 200 msec after said implementation of repeated polarization pulse was adopted as the trail current. The effect of each compound on the hERG current was evaluated in terms of a deviation ratio of the trail current to the prior trail current that was observed before the start of this assay. The average deviation ratio (%) of the post hERG current to the prior hERG current of each compound was calculated, and the inhibition ratio of each compound on the hERG current was determined by deducing the average deviation ratio of the negative control compound (Nicorandil) as run-down. Thus the compounds having a ratio of more than 20% in terms of the hERG current inhibition were judged as positive in respect to the hERG current inhibition.

Results

As can be seen from Table 4 below, all the compounds other than Compound 1 of the present invention (i.e., the prior art compounds) block the hERG potassium ion channel at a level ranging from 30 to 300 μM. This suggests that the presently claimed compounds are

prominently superior to any compounds described in the cited prior art in respect to antibacterial activities and the action on hERG current inhibition.

Table 4:

#	Chemical Structure(or Code)	Concentration (μM)	hERG current inhibition (means±SD (%) (n))	Inhibition Criteria (%) : 20	Judgement
Positive Control	E-4031	0.001	0.2±7.12 (4)		
		0.01	11.5±4.97 (3)	+	Inhibition from 0.1 μM
		0.1	80.8±11.23 (4)		
Negative Control	Nicorandil	0.3	-1.4±1.00 (4)		
		3	-3.8±5.51 (4)	-	No inhibition
		30	5.2±3.47 (4)		
10/572742 Compound 1		30	-4.4±3.11 (4)		
		100	7.2±5.10 (4)	-	No inhibition
		300	16.3±1.66 (4)		
US6625353 (Compound A) US6121285 (Example15)		30	41.7±10.44 (4)		
		100	45.9±2.04 (4)	+	Inhibition from 30 μM
		300	65.0±6.57 (3)		
US6121285 US6184388 Example 16		30	42.7±4.18 (4)		
		100	70.4±3.19 (4)	+	Inhibition from 30 μM
		300	88.7±3.52 (4)		
US6448266 Inventive Example 7		30	17.8±2.41 (4)		
		100	34.4±1.29 (4)	+	Inhibition from 100 μM
		300	32.7±1.77 (4)		
US6448256 Inventive Example 5		30	30.6±5.59 (4)		
		100	46.6±1.86 (4)	+	Inhibition from 30 μM
		300	62.6±3.21 (4)		
US6448266 Inventive Example 6		30	6.6±2.23 (4)		
		100	19.2±1.87 (4)	+	Inhibition from 300 μM
		300	32.0±15.99 (4)		

8. As discussed above, and as evidenced by Tables 3 and 4, the presently claimed compounds, characterized by having a cyano group at the position 8 thereof, have not only significantly excellent antibacterial activities but also appreciably high safety, compared with the compounds described in the cited prior art. Thus the claimed invention would have been obvious to one skilled in the art, by any of the cited references, or by any combination thereof.

9. I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

10. Further Declarant saith not

Hisashi Takahashi

Name:

Sep. 28, 2009

Date